Supplementary Data

Supplementary Methods

Cardiac progenitor cell culture

Cardiac progenitor cell (CPC) colonies that grew from single cells were evaluated by flow cytometry with antibodies against CPC markers (c-kit, MDR1, and Nkx2-5; Santa Cruz Biotechnology). Cells were cultured in Ham's F-12 media supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Hyclone), L-glutamine, penicillin/streptomycin (Invitrogen), 10 ng/mL leukemia inhibitory factor, and 10 ng/mL basic fibroblast growth factor.

Real-time polymerase chain reaction

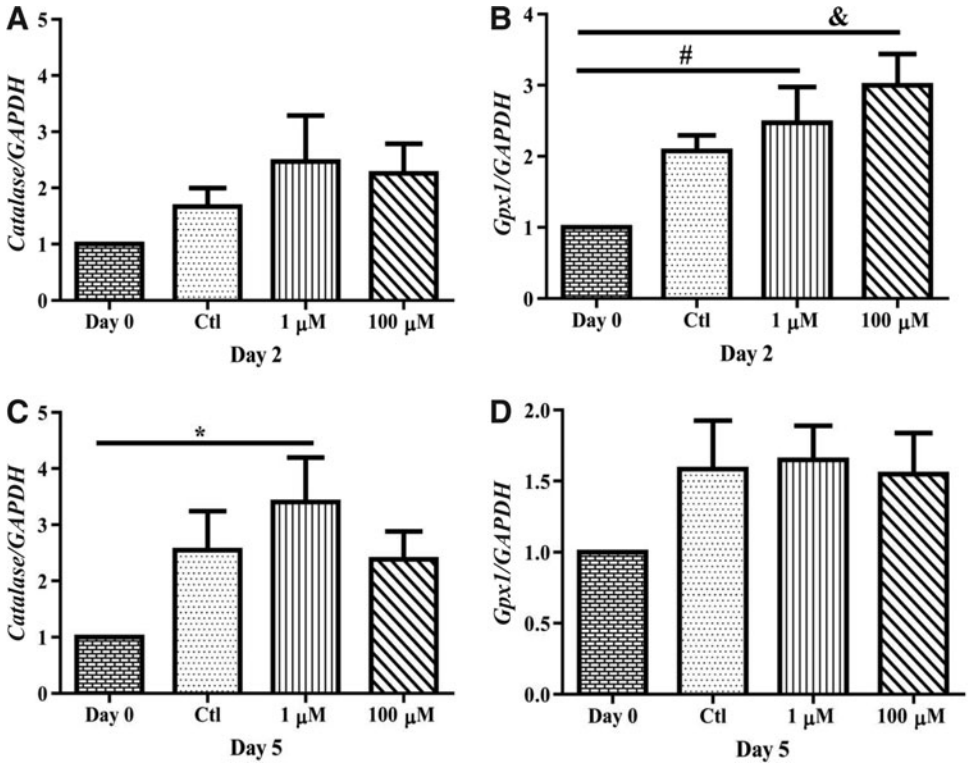
Gene expression was determined by real-time polymerase chain reaction (PCR) as follows: Cells were incubated with the Trizol reagent (Invitrogen) to extract mRNA according to the manufacturer's protocol. cDNA was generated by reverse transcribing the mRNA using moloney murine leukemia virus reverse transcriptase (Invitrogen), oligo dT, and random hexamers. Samples were run using the StepOnePlus machine (Applied Biosystems) with SYBR Green PCR master mix (Applied Biosystems) using primers for *catalase*, *glutathione peroxidase 1*, *thioredoxin 1*, *peroxiredoxin 6*, *troponin C*, *troponin T*, smooth muscle 22 actin (*Tagln*), alpha SM actin (*Acta2*), vascular endothelial growth factor 2 (*Kdr*), platelet endothelial cell adhesion molecule (*Pecam1*), *Gata4*, and *Nkx2-5* as listed in Supplementary Table 1. All target mRNA Ct values were normalized to GAPDH and expressed as fold changes using the ΔΔCt method.

Apoptosis staining

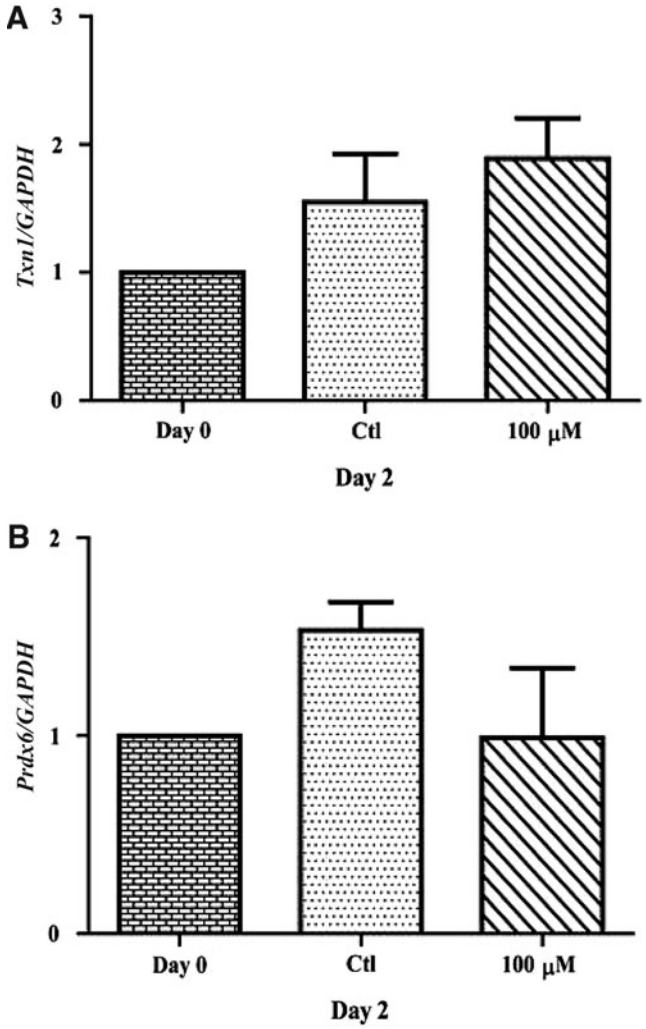
Media were collected and cells were dissociated from the plate with Trypsin EDTA (Invitrogen), and then cells were washed with phosphate-buffered saline and centrifuged at 1,250*g* for 5 min. As per the manufacturer's protocol (Invitrogen), cells were labeled with 5 μL of fluorescently tagged Alexa-647 antibodies in 500 μL of the Annexin V binding buffer [HEPES (10 mM), $CaCl_2$ (2.5 mM), NaCl (140 mM), and pH 7.4 buffer] for 15 min at 24°C. At completion of the incubation, 400 μL of the Annexin V binding buffer was added to the cells and the extent of apoptosis was evaluated using a Becton Dickinson LSR II Flow Cytometer (BD Biosciences). Results were analyzed using the FlowJo 7.6.5 software.

Invasive hemodynamics

Briefly, cardiac function was first assessed under anesthesia by small animal echocardiography (Vevo770R; Visualsonics).



SUPPLEMENTARY FIG. S1. Hydrogen peroxide (H_2O_2) scavenging enzymes are not regulated by H_2O_2. Cardiac progenitor cells (CPCs) were cultured in media with 1 or 100 μM H_2O_2 or without H_2O_2 replaced daily for 2 **(A, B)** or 5 **(C, D)** days and mRNA was harvested and gene expression levels were measured through reverse transcription (RT)-polymerase chain reaction (PCR). At 2 and 5 days, H_2O_2 treatment did not alter *catalase* **(A, C)** or glutathione peroxidase 1 (*Gpx1*) **(B, D)** gene expression. Data are mean ± SEM. $^{*}P < 0.05$, $^{\#}P < 0.01$, and $^{\&}P < 0.001$, $n = 4$–11; ANOVA followed by the Tukey–Kramer post hoc test.

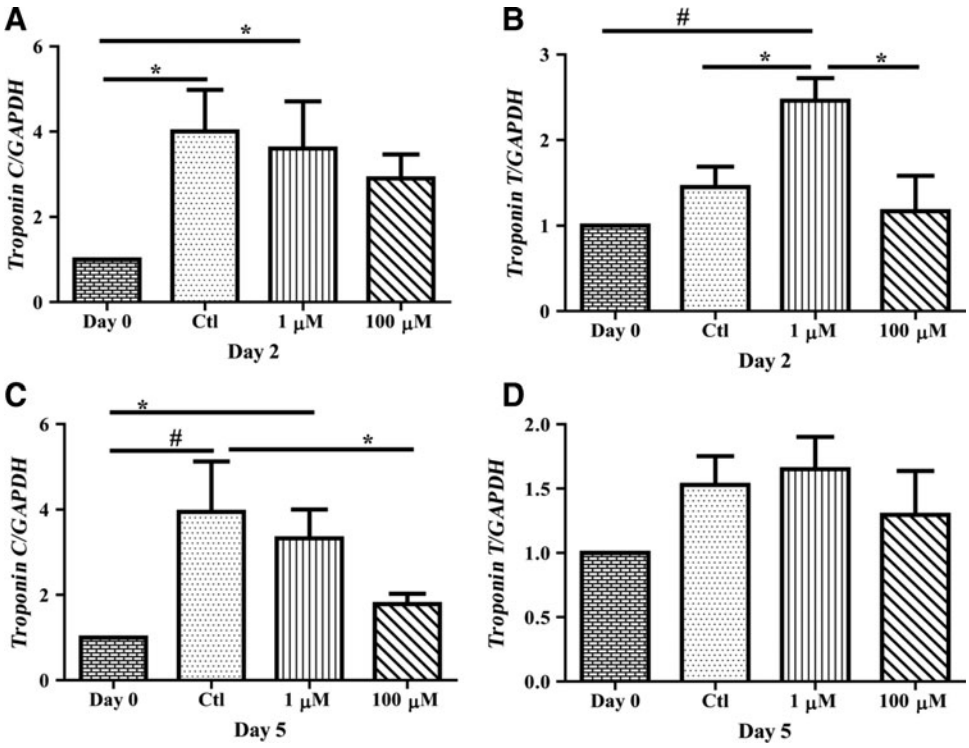


SUPPLEMENTARY FIG. S2. H_2O_2 scavenging enzymes are not regulated by H_2O_2. CPCs were cultured in media with 100 μM H_2O_2 or without H_2O_2 replaced daily for 2 **(A, B)** days and mRNA was harvested and gene expression levels were measured through RT-PCR. At 2 days, H_2O_2 treatment did not alter thioredoxin 1 (*Txn1*) **(A)** or peroxiredoxin 6 (*Prdx6*) **(B)** gene expression. Data are mean ± SEM. $n = 3$–4; ANOVA followed by the Tukey–Kramer post hoc test.

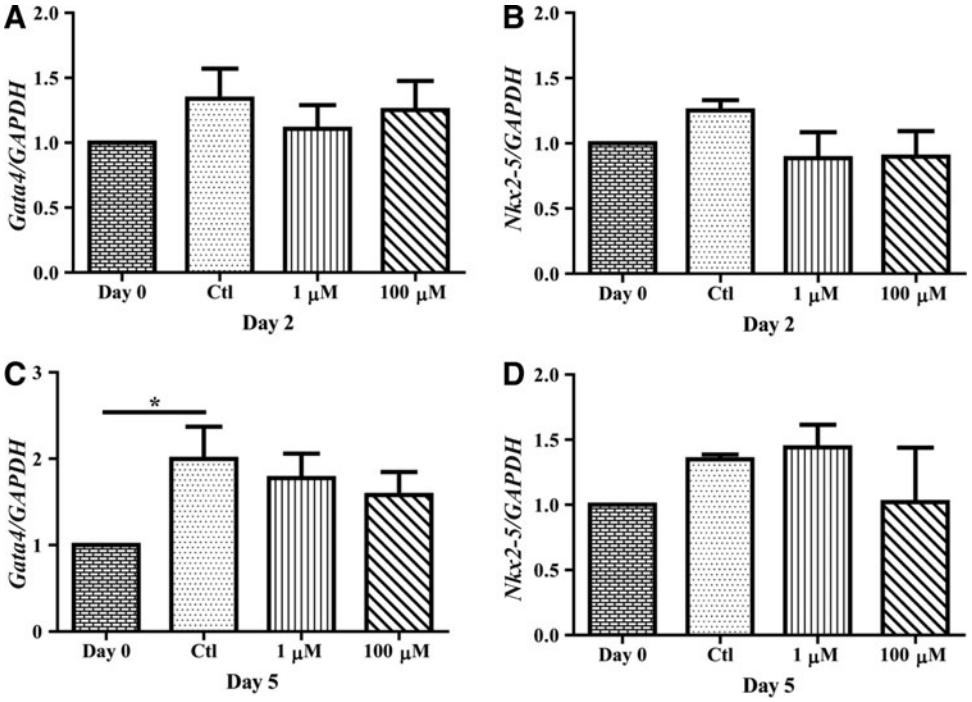
Short axis values of left ventricular end systolic and end diastolic dimensions were obtained and used for pressure–volume calculations. For invasive ventricular function measurements, animals were anesthetized with isoflurane and placed on a heating pad. The chest was opened and a 2-french conductance catheter (Milliar Instruments, MPVS Ultra and SPR-838) was introduced apically into the left ventricle. Pressure–volume loops were acquired with a PowerLab data acquisition system and analyzed using the LabChart software package (Millar). The catheter was calibrated according to the manufacturer's instructions. At the completion of the experiment, animals were euthanized and hearts were excised for fixation and embedding.

Immunohistochemistry

Brightfield images were taken with a Zeiss Axioskop 2 microscope equipped with an Axiocam CCD camera using a 2.5× objective. Overlapping sections were made so that images could be stitched together for reconstitution of the entire heart. Cardiac tissue sections were also probed with an isolectin Alexa-568 conjugate (Invitrogen) for endothelial cells, and an antibody against firefly luciferase (Abcam) for implanted CPCs and visualized with an appropriately Qdot655 conjugated secondary antibody (Life Technologies). Nuclei were stained using 4′,6-diamidino-2-phenylindole (Molecular Probes). Images were taken with a confocal Zeiss LSM 510 META Laser Scanning Confocal microscope using a 40× objective for vessels and a 63× objective for endothelial cells.



SUPPLEMENTARY FIG. S3. Troponin isoforms show minimal regulation by H_2O_2. CPCs were cultured in media with 1 or 100 μM H_2O_2 or without H_2O_2 replaced daily for 2 **(A, B)** or 5 **(C, D)** days and mRNA was harvested and gene expression levels were measured through RT-PCR. At 2 days, only *Troponin T* was significantly elevated during CPC differentiation in the presence of 1 μM H_2O_2 compared to a time-matched untreated control. Chronic (5 days) 100 μM H_2O_2 treatment inhibited *Troponin C* gene expression below that of untreated cells. Data are mean ± SEM. $*P < 0.05$, $\#P < 0.01$, $n = 4$–10; ANOVA followed by the Tukey–Kramer post hoc test.



SUPPLEMENTARY FIG. S4. Early markers of cardiogenesis were not affected by H_2O_2 treatments. CPCs were cultured in media with 1 or 100 μM H_2O_2 or without H_2O_2 replaced daily for 2 **(A, B)** or 5 **(C, D)** days and mRNA was harvested and levels were measured through RT-PCR. *Gata4* mRNA expression increased over time during differentiation. At 2 and 5 days, H_2O_2 treatment did not alter *Gata4* or *Nkx2-5* gene expression. Data are mean ± SEM. *$P < 0.05$, $n = 3$–11; ANOVA followed by the Tukey–Kramer post hoc test.

Supplementary Table S1. Primers Used for the Study

Gene	*Forward primer*	*Reverse primer*
Gpx1	5′AGTTCGGACATCAGGAGAATGGCA3′	5′AGGCATTCCGCAGGAAGGTAAAGA3′
Catalase	5′TTGACAGAGAGCGGATTCCT3′	5′GGCATCCCTGATGAAGAAAA3′
Troponin T	5′ AAGGCCAAAGTCACCGGGCG3′	5′ TCGGGTGCCTGGCAAGACCT3′
Troponin C	5′GATCTCTTCCGCATGTTTGACA3′	5′TGGCCTGCAGCATCATCTT3′
Kdr	5′GCCAATGAAGGGGAACTGAAGAC3′	5′CTGGCTGCTGGTTATGCTGTC3′
Pecam1	5′CGGCAAAGTGGTCAAGAGAAGCAA3′	5′TGGGTGCAGTCCCATTTACTGACT3′
Acta2	5′CCCAGATTCAGGAACAGCAT3′	5′GTTAGCAAGGTCGGATGCTC3′
Tagln	5′AGCCAGTGAAGGTGCCTGAGAAC3′	5′TGCCCAAAGCCATTAGAGTCCTC3′
Gata4	5′TCAAACCAGAAAACGGAAGC3′	5′GCATCTCTTCACTGCTGCTG3′
Nkx2-5	5′CGCCCTTCTCAGTCAAAGAC3′	5′GAGCTACTGTCAGGCTTGGG3′
Txn1	5′GTGAAGCTGATCGAGAGCAA3′	5′TCCTTGTTAGCACCAGAGAAC3′
Prdx6	5′CCTGGAGCAAGGACATCAAT3′	5′GGAGTCAACCACTCTGAGAATC3′
GAPDH	TaqMan VIC MGB 4352338E	